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Hematopoiesis in Conventional Mice After Wound Trauma[1]

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Macrophages, granulocytes, and platelets serve in wound debridement, bacterial neutralization, and homeostasis. Replacement of such cells may involve perturbations in the clonogenic cell populations responsible for replenishing specific adult cell populations. For example, the human blood granulocyte progenitor cell compartment is increased following abdominal hysterectomy (Philip et al, 1980). In mice, skin wound trauma produced changes in the proliferative cell compartments of the hematopoietic tissues 24 hrs after injury (Ledney et al, 1980). In this study we report (1) some of our findings on the hematopoietic clonogenic cell changes in conventional mice after trauma and (2) circulating substances which may mediate these changes.

Materials and Methods

Animals: Groups of 10-20 week old female B6CBF1 Cum BR mice were wounded under Metafane anaesthesia between the hours of 10AM and 2PM. A non-lethal 2.5 cm^2 circular wound (4% skin surface) was cut in the anterior-dorsal skin fold

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and underlying panniculus carnosus muscle (between the shoulder blades) with a steel punch. The punch was cleaned after the wounding of each animal by immersion in 70% ethanol. Wounds were untreated and left open to the environment. After injury, all mice were placed in sanitized cages with autoclaved hardwood chip bedding. Control mice were anaesthetized and handled in the same manner as the wounded animals except for the wounding procedure.

Clonogenic Cell Assays: Colony forming unit-spleen assays (CFU-s) were used to determine the pluripotent cell numbers in hematopoietic tissues. Experimental groups (6-8 mice) were exposed to 10 Gy ^{60}Co radiation (dose rate 0.4 Gy/min). Within 4 hrs of irradiation, each mouse was injected i.v. with either 25 x 10^4 spleen or 25 x 10^3 marrow cells derived from control (C) or wounded (W; 3,7,10 or 14 days post-wounding) donors. Additional irradiated mice received 1,2, or 5 x 10^5 isolated peripheral blood cells from C or W (day 3) donors. Spleens were removed at 8 days, fixed in Bouin's solution (2-4 hr) and surface colonies counted.

Endogenous CFU (E-CFU) studies were performed on groups of mice wounded 24 hr prior to radiation (7 or 9 Gy) exposure. Spleen colonies were counted at 5,7,10 and 12 days after irradiation.

Tissues containing granulocyte-macrophage colony forming cells (GM-CFC) and macrophage colony forming cells (M-CFC), were taken from C or W mice (days 3,7,10 or 14 post-wound). 10^6 spleen, 25 x 10^3 marrow, and 0.5, 1 or 2 x 10^5 nucleated peripheral blood cells were grown in triplicate double-layer agar cultures with 5% growth stimulator (pregnant mouse uterine extract). Colonies (>50 cells) were counted at day 10 (GM-CFC) and day 25 (M-CFC).

All clonogenic data are quantitated on the basis of 10^6 nucleated cells.

Circulating Mediators: Serum from C or W mice (days 2 & 3) was examined for colony stimulating activity (CSA) via M-CFC and uM-CFC assays. C-reactive protein (C-RP) was measured nephelometrically using human C-RP antisera and standards. Plasma prostaglandin E_2 (PGE_2) levels were determined by ^{125}I-radioimmunoassay of samples previously subjected to organic extraction and purification on C-18 columns. C-RP and PGE_2 samples were obtained from C and W mice days 1-7,9,11,14,17 and 21.

Results

Clonogenic Cells After Wound Trauma: Splenic CFU-s in W mice were increased 2-fold on day 3 after injury. Also at this time, peripheral blood of W mice contained 4-fold more CFU-s (71±4) than C mice (16±4). No significant changes were seen in femoral marrow CFU-s.

The splenic GM-CFC concentration was increased 5-fold 3 days after trauma (80±4 vs 16±2). Thereafter, a 1.5-2-fold increase in splenic GM-CFC was observed during the remainder of the 2-week wound healing period. In the femoral marrow, the GM-CFC concentration was increased only on day 3 after trauma (1870±139 vs 1328±116). Also on day 3, an 8-fold increase in the GM-CFC concentration in the peripheral blood (48±11 vs 6±2) was seen.

The splenic M-CFC compartment of W mice was reduced 30-70% from that of C mice (153±9) during the 2-week healing period. After injury, the femoral marrow concentration of M-CFC was increased on day 14 only (3224±403 vs 2136±157). The peripheral blood concentration of M-CFC was similar in both W and C mice (130-160) on day 3.

Wounding 24 hr prior to 7 Gy resulted in 3-fold more E-CFU at 5,7, and 10 days after irradiation than that formed after 7 Gy only (control E-CFU were 0.8, 1.7, and 6.4 respectively). A 10-fold increase in E-CFU at 5, 7, and 10 days was seen when wounding preceded 9 Gy by 24 hr (control E-CFU were 0.2, 0.4, and 2.7 respectively. Thus wounding had a greater effect at 9 Gy ($p<.05$) than at 7 Gy. The doubling time for E-CFU was 1.2 days and was independent of the radiation dose or the combined injury.

Circulating Mediators: CSA was detected in the serum of mice 2-3 days after trauma. About 250 GM-CFC and 20 M-CFC normal marrow cells cloned in 0.1 ml of serum from W mice. No colonies were formed in the serum from C mice while approx. 1200 and 200 GM-CFC and M-CFC respectively, cloned in enhancing medium.

Serum C-RP concentration increased 50-300% 2-4 days after wound trauma. One week after wounding the C-RP level returned to the control level of 4.0 ug/ml.

Plasma PGE_2 concentration increased immediately after trauma and was increased 10-20-fold 2-3 days after injury. By one week, PGE_2 returned to control values of about 50 pg/ml.

Discussion

In conventional mice, the pathophysiological stimulus of skin wounding and subsequent healing results in significant perturbation in clonogenic cell populations and circulating substances that may mediate hematopoiesis. Our findings suggest that tissue injury provokes cellular and humoral responses in the host's attempt to maintain homeostasis. The utilization of granulocytes to clear cellular debris and bacteria from the wound site may result in the mobilization of proliferative cells from the bone marrow and spleen. These cells produce mature elements to assist in repair. As the mature cells are consumed, the demand for more mature cells is met by proliferation and amplification of progenitor cell compartments (GM-CFC and M-CFC), the less differentiated E-CFU compartment, and the pluripotent CFU-S compartment.

One focus of our work is to identify mediators which may regulate clonogenic cells after trauma. PGE_2 is a known modulator of hematopoiesis (Kurland and Moore, 1977) and cells in resting stage (G_o) are known to undergo proliferation when exposed to higher levels of PGE_2 (Williams, 1979) and this could explain the reduced splenic M-CFC seen after trauma. Additionally, there is *in vitro* evidence that C-RP inhibits M-CFC expression (Marcelletti et al, 1982). The finding of a serum substance (CSA) capable of promoting GM-CFC growth suggests negative feedback regulation (consumption of granulocytes) evoked by trauma. But, *in vitro* demonstration of a CSA effect does not mean that it is active *in vivo*.

The hematopoietic proliferation and attending mediator release may be the result of host responses to infection or endotoxins. However, abscesses, pus formation, lymph node involvement, and histologic organ involvement with bacteria were never seen. Also, a secondary peak in C-RP, indicative of infection (Rowe et al, 1984) was never found. However, all wounds were contaminated with one to three naturally occurring skin or alimentary tract bacteria. Wound colonization with specific bacteria may enhance healing (Levenson et al, 1983) and/or inhibit colonization with more desirable organisms (Papageorgiou et al, 1976).

Bacterial endotoxin is a known modifier of hematopoietic responses. This substance could be released by bacteria into circulation at the wound site or through disrupted intestinal cell tight junction barriers after injury (Walker and Porvaznik, 1983) Germ-free animals may

provide a clue to understanding the clonogenic and mediator substances released after trauma. In germ-free rats, the intensity of the inflammatory reaction is less than that of rats with a "normal" flora (Dorati et al, 1971). Future experiments measuring hematopoietic proliferation and mediator substance release in germ-free animals are planned.

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